EFFECT OF ANTI INFLAMMATORY IRRIGANTS ON INFLAMMATORY MEDIATORS IN ROOT CANAL TREATMENT OF SYMPTOMATIC VITAL TEETH-IN VIVO STUDY.

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THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY

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BRANCH IV

CONSERVATIVE DENTISTRY AND ENDODONTICS

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DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation titled **"EFFECT OF ANTI INFLAMMATORY IRRIGANTS ON INFLAMMATORY MEDIATORS IN ROOT CANAL TREATMENT OF SYMPTOMATIC VITAL TEETH-IN VIVO STUDY."** is a bonafide and genuine research work carried out by me under the guidance of **Dr.I.Anand Sherwood, M.D.S.,PhD Professor and Head** , Department of Conservative Dentistry and Endodontics, CSI college of dental sciences and research, Madurai

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This dissertation is submitted to **THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY**, in partial fulfillment for the degree of **MASTER OF DENTAL SURGERY – CONSERVATIVE DENTISTRY AND ENDODONTICS, BRANCH IV**. It has not been submitted (partial or full) for the award of any other degree or diploma.

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CONTENTS

LIST OF TABLES

LIST OF GRAPHS

LIST OF FIGURES

12	**SAMPLES ARE STORED IN EPPENDORF TUBE WITH PHOSPHATE BUFFERED SALINE**	**33**
13	**SAMPLES STORED IN EPPENDORF TUBE**	**33**
14	**SAMPLES TAKES AFTER BIOMECHANICAL PREPARATION**	**34**

LIST OF ABBREVIATIONS

S.NO	ABBREVIATIONS	DESCRIPTIONS
1	NaOCL	Sodium hypochlorite
2	EDTA	Ethylenediaminetetraacetic acid
3	GP	Gutta Percha
4	IL-8	Interleukin 8
5	SP	Substance P
6	PMNs	Polymorphonuclear neutrophils

INTRODUCTION

INTRODUCTION

Pulpal disease and concomitant pain is caused by caries, trauma, or as a result of restorative procedures. Onset of pain subsequent to caries varies depending on a number of factors including presence of prior restorations, individual pain thresholds and host inflammatory and immune resistance. The major cause of pain is due to release of inflammatory mediators that activate sensitive nociceptors surrounding the tooth. An increase in vascular permeability and the migration of leukocytes from blood vessels can be observed during the first moments of the inflammation process, due to the presence of inflammatory substances in the inflamed site. Normally when fine afferent C and A δ fibers are activated by brief, high intensity stimuli which induce little or no tissue damage, transient pain is induced and serves as a physiological warning[1]. During the inflammation produced by mild tissue damage or infection, afferent fibers are activated by lower intensity stimuli and the pain produced differs in quality and may be more persistent[1]. Several important factors are associated with inflammation besides the stimulation of peripheral nerve fibers to induce pain there are changes in local blood flow and vascular permeability, activation and migration of immune cells and changes in the release of growth and trophic factors from surrounding tissues[1]. In dental pulp, periodontal ligament inflammation has a neurogenic source which is induced by the release of neuropeptides from periapical tissue C type nerve fibers, after being injured during root canal treatment.

Interleukin-8 (IL-8) is a member of the chemokine C-X-C subfamily that displays potent chemotactic activities for human neutrophils and T lymphocytes[2]. IL-8 is a potent chemokine with strong chemoattractive activity for neutrophils. IL-8 stimulates neutrophils to a higher state of activation. It is rapidly synthesized at local sites of

inflammation where it fulfils its function of recruiting and activating acute inflammatory cells[2]. In diseased pulps, IL-8 was reported to be produced by pulpal inflammatory cells and endothelial cells in addition to odontoblast[2]. In addition, bacterial lipopolysaccharide stimulated pulp fibroblasts produce higher levels of IL-8 than the unstimulated group[4]. IL-8 was originally isolated from the culture supernatants of stimulated human blood monocytes.This chemokine can also be generated by a variety of cells, including fibroblasts, lymphocytes, hepatocytes, epithelial cells, and endothelial cells[2]. Cytokines are generally excellent markers of inflammation. Cytokines, which are polypeptides secreted by leucocytes and other cells, act as modulators of immune and inflammatory responses and can be divided into inflammatory and anti-inflammatory cytokines[2].

Levels of IL-8 were significantly higher in pulpal tissue from irreversible pulpitis as well as pulpal blood[4].There is an increase in mRNA level of IL-8 in symptomatic teeth compared with healthy teeth[5]. IL-8 was detected in pulps with irreversible pulpitis and caries exposure but was not detectable in normal pulps. Irreversible pulpitis pulps have 4-fold higher median IL-8 levels than asymptomatic caries exposure pulps[2]. IL-8 may be a good contender for a marker for the degree of pulpal inflammation and appears to be correlated with pulpal symptoms[2].

Substance P is a neuropeptide produced a subset of capsaicin sensitive sensory peripheral neuron cell bodies localized in dorsal root and trigeminal ganglia which plays a pivotal role in the transmission of noxious stimuli in spinal cord [6]. The stimulation of capsaicin sensitive, sensory peripheral terminal of the neurons results in the peripheral release of substance P [6]. In the central pulp, SP fibers traverse in close proximity to blood vessels. In the periphery, many SP fibers are directly associated with small blood vessels[7]. SP is released by neurons on various types of noxious

stimuli. The types of stimuli are noxious, thermal, mechanical, and chemical stimulation of the pulpodentin complex. The electrical stimulation of thin, unmyelinated afferent C fibers or the administration of capsaicin has been found to result in the release of SP[7].The biological effects of released SP are induced following its binding to specific G protein-coupled NK receptors. There are three types of tachykinin receptors, NK1, NK2, and NK3 exhibiting preferences for substance P, neurokinin A, and neurokinin B[6]. Substance P primarily acts on NK1 receptors and simulation of the NK1 receptor induces several second messengers systems[6]. Two dynamic changes occur to peptidergic afferent neurons during inflammation. First, there is a sprouting of afferent fibers as based by both anatomic and receptor field. Second, local increases in inflammatory mediators trigger neuropeptide release, leading to neurogenic inflammation, including vasodilation, plasma extravasation, and recruitment/regulation of immune cells such as macrophages, mast cells, and lymphocytes[7]. SP induces interleukin-8 secretion from human dental pulp cells and enhances expression of lipopolysaccharide-induced inflammatory factors in dental pulp cells[8].

Ketolorac tromethamine is a member of the pyrrolo pyrrole and its primary mode of action is the inhibition of the cyclooxygenase (COX-1) pathway that metabolises arachidonic acid to prostaglandins and thromboxanes[9]. Is a potent and available both in oral and injectable forms. In 1999 Rogers et al used ketorolac as an intracanal medicament it has a better pain relief than other drugs[12].

Dexamethasone is a steroidal antiinflammatory drug (SAID) that inhibits phospolipase-A2 and consequently reduces prostaglandin and leukotriene synthesis, decreasing polymorphonuclear leukocyte chemotaxis. It also suppresses the production of free oxygen radicals and nitric oxide by endothelial cells. SAIDs are

able to down-regulate many proinflammatory cytokines involved in the inflammatory process and immune response[10]. In 2007 Athanassiadis B et al concluded that the effectiveness of corticosteroids in reducing periapical inflammation and the incidence of pain following root canal preparation[28].

AIM AND OBJECTIVES

AIM AND OBJECTIVES

To observe the influence of Ketorolac tromethamine and Dexamethasone root canal irrigants on the expression of IL-8 and substance P in pulp and periapical tissues following single visit root canal treatment in teeth with symptomatic irreversible pulpitis. Secondary objective was to evaluate the incidence of post operative pain following usage of different irrigating solutions.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Wade K Nobuhara et al (1993)[11] compared the anti-inflammatory effects of dexamethasone on periapical tissues following enodontic overinstrumentation**.** A total of 36 sprague dawley rats were used in this study.,the animals were 5 week old first the administration of anesthetic agent by intramuscular injection. The occlusal surfaces of the mandibular left and right first molars were swabbed with Betadine and the pulp chambers were surgically exposed with a #1 HP bur in a slow-speed handpiece. In half of the rats vital group endodontic over instrumentation was performed immediately following exposure of the pulp chamber. In the remaining rats nonvital group the pulp chambers were left exposed to the oral environment for 7 days prior to endodontic over instrumentation to produce partial necrosis of the pulp tissue.after all the preparations performed. The rats were killed at observation periods of 6, 24, and 48 h following endodontic over instrumentation. At each observation period, six rats from the vital group and six rats from the nonvital group were anesthetized and killed. The results showed that in the vital group, the saline controls demonstrated an increase in the number of PMNs per square millimeter in the apical and middle regions of the PDL space throughout the observation period.. Both in vital and nonvital group after 48 hrs the average number of PMNs per square millimeter in the apical and middle regions of the PDL space was significantly lower by buccal infiltration of dexamethasone.

Martin J Rogers et al (1999)[12] compared the effect of intracanal use of ketorolac tromethamine and dexamethasone with oral ibuprofen on post treatment endodontic pain a total of 48 patients were selected with vital pulp. The root canal appointments were performed in two appointments first cleaning and shaping**.** And the patients were asked to evaluate their pretreatment pain with a visual analog scale. The post

treatment pain was evaluated at 6, 12, 24, and 48 hours. The results showed that at the 12 hour period both dexamethasone and ketorolac provided statistically significant better relief than placebo. At 24 hour period only ketorolac demonstrated better pain relief than placebo.

George T J Huang (1999)[3] studied that increased interleukin-8 in inflamed human dental pulps. Study subjects (selected patients) were in good health and were not receiving long-term anti inflammatory medication. Normal pulpal tissue was obtained from freshly extracted,intact, caries-free third molars. Immediately after extraction the teeth were stored in normal saline solution. Under water coolant, each tooth was grooved longitudinally with a fissure bur at high speed. The tooth was then split, and with the entire pulp (coronal and radicular) elevated with cotton pliers, maximum pulpal tissue was obtained. Samples for the immunohistochemical analysis (ELISA) were immediately either embedded in O.C.T. compound snap-frozen in liquid nitrogen, and stored at –70°C until the preparation of frozen sections or placed in 10% phosphate-buffered formalin. The tissue recovered during pulpectomy after the chamber was carefully unroofed, coronal pulp was excised with a sharp endodontic spoon excavator and then stored. Results showed that the normal pulps, 53% (8/15) showed detectable but very low levels of IL-8, whereas 71% (10/14) of samples from diseased pulps demonstrated detectable and elevated levels of IL-8. In diseased pulps IL-8 is mainly produced by pulpal inflammatory cells, endothelial cells in addition to odontobasts.

Javier Caviedes-Bucheli et al (2008)[7] reviewed neuropeptides in dental pulp. The term neurogenic inflammation has been developed to describe the component of inflammation caused by an appropriate stimulus applied to peripheral neurons, resulting in the release of neuropeptides that alter multiple processes including

vascular permeability and vasodilation at the site of injury. Neuropeptides are defined as peptide neurotransmitters or neuromodulators. the relative abundance of SP positive immunoreactive nerve fibers within the tooth pulp and dentin of several species, including human . In the central pulp, SP fibers traverse in close proximity to blood vessels. In the periphery, many SP fibers are directly associated with small blood vessels. Two dynamic changes occur to peptidergic afferent neurons during inflammation. First, there is a sprouting of afferent fibers as based by both anatomic and receptor. Second, local increases in inflammatory mediators trigger neuropeptide release, leading to neurogenic inflammation, including vasodilation, plasma extravasation, and recruitment/regulation of immune cells such as macrophages, mast cells, and lymphocytes. Activation of the sympathetic nervous system reduces pulpal blood flow via local release of neurotransmitters in the pulp, including norepinephrine, NPY, and adenosine triphosphate, which constrict vessels expressing the correspondent receptor. Both SP and CGRP are released from terminals of pulpal nociceptors consisting of unmyelinated C fibers and thinly myelinated A-delta fiber. SP and CGRP are potent vasodilators in the dental pulp, whereas NKA has a much smaller effect on pulpal blood flow . On activation of sensory nerves, either by brief antidromic electrical stimulation of the inferior alveolar nerve or by direct simulation on the tooth crown, neuropeptides are released, inducing a long-lasting blood flow increase and increased vascular permeability in the pulp . The initial component of the vasodilator response is mediated by SP, whereas the continued long-lasting rise in blood flow is dependent on CGRP. After exerting their effects, neuropeptides are rapidly degraded by enzymes in the pulp tissue.

Alessandra Cecilia Oliveria Siliva et al (2009)[4] studied interleukin-1 beta and interleukin-8 in healthy and inflamed dental pulps. Twenty pulp tissues (10 healthy

pulps and 10 inflamed pulps) were obtained from patients who had teeth indicated for extraction (for the healthy pulps) or pulpectomy (for the inflamed pulps). After the extraction, healthy third molars were placed in 4% paraformaldehyde solution and immediately prepared with a longitudinal 1 mm depth sulcus, made with a diamond disc in low speed hand piece. After this preparation, a chisel was inserted in the sulcus, and the teeth were cleaved, in order to allow the collection of the unharmed pulp tissue. Tissues were placed into a new 4% paraformaldehyde solution, in 0.1 M phosphate buffer at 4º C, for 24 h. immunohistochemical reactions , ELISA and cell culture.results showed that the inflamed pulps were strongly stained for both cytokines in inflammatory cells, while healthy pulps were not immunolabeled. ELISA from tissues quantitatively confirmed the higher presence of both cytokines. Additionally, cultured pulp fibroblasts stimulated by LPS also produce more cytokines than the control cells. And it was concluded that the inflamed pulps present higher amounts of IL-1ß and IL-8 than healthy pulps and that pulp fibroblasts stimulated by bacterial LPS produce higher levels of IL-1ß and IL-8 than the control group.

Marcia Thais Pochapski et al (2009)[9] analyzed the effect of pretreatment of dexamethasone on post endodontic pain. Fifty patients (26 men and 24 women) between the ages of 18 and 67 years (mean age 42.1 years) were selected Dexamethasone group-(73%) has low levels of endodontic pain than placebo group-(33%). The groups - were randomly divided into 2 experimental groups: group 1, placebo; and group 2, dexamethasone (4 mg). Both medications were administered 1 hour before the conventional root canal therapy. Each patient was anesthetized with a solution of 2% mepivacaine with 1:100,000 epinephrine. The canals were enlarged to the size of the #25 file or larger (depending on the size of the canal), 1.0-1.5 mm short of the radiographic apex. Copious irrigation with 2.5% sodium hypochlorite and 17%

liquid EDTA was used between each file, with the irrigant remaining in the canal throughout the entire procedure. When instrumentation was completed, the canals were rinsed thoroughly and dried with paper points; they were then filled with calcium hydroxide paste. A cotton pellet was placed in the access cavity, which was restored with intermediate restorative. Patients were instructed to complete a pain diary after 4, 12, 24, and 48 hours of root canal instrumentation. Results showed that postendodontic pain showed a statistically significant difference between groups at 4 and 12 hours. Dexamethasone treatment was associated with the lowest levels of endodontic pain; however, no statistical difference was observed at 24 or 48 hours. The percentage of subjects reporting no pain after a 4-hour period was 73% for the dexamethasone group and 33% for the placebo group. After a 12-hour period, 85% of patients in the dexamethasone group and 52% of patients in the placebo group reported no pain. After the 24 and 48 hour periods, no pain was observed in 90% of the patients in both groups .

Javier Caviedes Bucheli et al (2010)[13] described the effect of three different rotary instrumentation systems on substance P and calcitonin gene related peptide expression in human periodontal ligament. Fifty periodontal ligament samples were obtained from 50 lower premolars in which extraction was indicated for orthodontic reasons. All teeth used were caries and restoration free with complete root development determined both visually and radiographically, without signs of periodontal disease or traumatic occlusion and without orthodontic forces. Teeth had only one straight canal (canal curvatures over 20˚ were not included). Teeth were equally divided and randomly assigned into the following five groups: (1) ProTaper Universal, (2) RaCe, (3) Mtwo, (4) hand instrumentation, and (5) intact-teeth control group. All teeth were anesthetized by an inferior alveolar nerve block injection of 1.8 mL 4% prilocaine

without vasoconstrictor. Root canal preparations were performed. Files were used only one time and then they were discarded, and the preparation time did not exceed 10 minutes for each tooth. Apical patency was verified in all groups with a #10 K-file. Canals were irrigated with 1.5 mL of 5% sodium hypochlorite between each file with a Monojet syringe with a 30-G needle placed 3 mm short of the working length. Teeth were extracted 10 minutes later after ending canal preparation with conventional methods without excessive injury to periodontal ligament. After extraction, a #10 K-file was placed into the canal until its tip protrudes from the foramen to corroborate apical patency and that all working lengths were at 0.5 mm from the foramen. Periodontal ligament samples were obtained from the apical 3 mm of the root with a periodontal curette, placed on an Eppendorf tube, snap frozen in liquid nitrogen, and kept at _70˚C until use. And the results showed that the neuropeptides were present in all periodontal ligament samples. Greater SP and CGRP expression found in Protaper universal group and followed by hand instrumentation, Race, M2 groups.

Paola Sacredote et al (2012)[6] in his article titled "Peripheral Mechanisms of Dental Pain: The Role of Substance P" reviewed that the onset of pain and inflammation is increased . SP is abundantly contained in the fibers that innervate the dental pulp and dentin. The production and release of SP molecules is highly increased in-noxious , thermal, mechanical, and chemical stimuli of pulp and periodontal ligament.

Elsalhy M et al (2012)[2] showed cytokines as diagnostic markers of pulpal inflammation. 108 subjects (57 male and 51 female) were participated in this study. The normal pulp group consisted of 25 teeth from 12 male and 13 female subjects with ages ranging from 18 to 35 years. The asymptomatic caries-exposed pulp group consisted of 40 teeth from 21 male and 19 female subjects with ages ranging from 18 to 54 years. The irreversible pulpitis group consisted of 43 teeth from 24 male and 19

female subjects' ages ranging from 18 to 60 years. After the tooth was isolated with a rubber dam, caries was removed, and the pulp was exposed with an excavator and/or a low-speed round bur. Blood from the exposed surface of the pulp was collected with a sterile cotton pellet. The pellet was held at the exposed site for 45–60 s to allow absorption of the pulpal blood. The pellets were then placed in 1 mL saline in heparin-coated tubes. Samples were stored at _20 °C until they were tested. the samples were centrifuged twice at 12 000 g for 10 min at 4 °C. The cotton pellets were then removed after which the samples were tested for cytokine levels by ELISA. Results showed that the levels are significantly higher in IL-6, IL-8, IL-10, TNF-α were detected in carious exposed pulps and irreversible pulpitis compared to normal teeth.IL-8 was higher in irreversible pulpitis compared to carious exposed teeth.

Kathleen G Neiva et al (2015)[14] in an investigation involving with patients presented for root canal therapy with history of traumatic tooth injury within two weeks. All patients were considered healthy according to the medical history questionnaire (n=43). Ancontrol group consisted of pulpal samples collected from intact teeth extracted for orthodontic reasons (n=10). Immediately after endodontic access and prior to any irrigation, dental pulp content was collected by placing one PeriopaperTM strip. in the chamber for 15 seconds. The strips were immediately placed in Eppendorf tubes and stored at -80°C until all the samples were ready to be processed. The samples were then eluted in 200 microliters of PBS and protein concentration was determined using BCA assay. For the control group, samples were collected from the pulp immediately after extraction. Results showed that avlusion experienced higher expression of SP than subluxation and lateral luxation in necrotic pulpal diagnosis. In the pulpal diagnosis SP and neuropeptides is affected and higher in symptomatic irreversible pulpitis.

Hazal Bicakci et al (2016)[15] studied the effect of rotary instrumentation with continuous irrigation upon pain and neuropeptides 40 patients participated in the present study.in mandibular premolar teeth with symptomatic pulpitis and symptomatic apical periodontitis. Each subject's pain level (spontaneous pain and pain on percussion test) was recorded by using the VAS score before the beginning of treatment procedure. GCF was collected from the experimental tooth at the beginning and 3 days after treatment. Before sample collection, the tooth was isolated with cotton rolls and dried gently with air. A saliva ejector was also used to prevent saliva contamination. Paper strips were inserted to the gingival sulcus until mild resistance was felt. The strips were removed from the gingival sulcus after 30 seconds. Two samples were collected for each tooth from gingival sulcus on mesial and distal vestibular surfaces. The samples contaminated with bloody saliva were discarded. The amount of collected GCF was determined by Periotron 8000. Then, all the strips were immediately placed in Eppendorf tubes and stored in the refrigerator at _40˚C for further analysis. The study correlates that various experimental and clinical pain measurement procedures compares conventional root canal preparation with continues irrigation. And the results showed that here it has not been more effective than standard preparation for reducing pain. CGRP and IL-10 levels were correlated in percussion pain and the levels were taken from the gingival crevicular fluid.

Marjorie Zanini et al (2017)[16] in a systematic review on inflammatory mediators concluded that IL-8 matrix metalloproteinase 9, TNF-α and receptor for advanced glycation end products expression increase at both the gene and protein levels during inflammation. IL-8 is produced by several pulpal cells including macrophages, lymphocytes, fibroblasts, and endothelial cells. In invitro conditions odontoblast cells exhibit low level IL-8 expression that significantl increases with pathogen-associated

molecular patter stimulation, especially lipopolysaccharide. This increased IL-8 expression is correlated with increased polymorphonuclear neutrophils (PMNs) within the pulp because IL-8 induces neutrophil chemotaxis and release of degradation enzymes during degranulation. Thus, IL-8 is often described as the primary regulatory molecule in the acute inflammatory response, and elevated levels may perpetuate and exacerbate the acute inflammatory response.

Christine Y Yu and Paul V Abbott (2018)[17] reviewed that endodontic treatment not only controls the bacterial infection, but also reduces the concentration of inflammatory mediators, the density of nociceptors and the tissue pressure in the confined pulp microenvironment. The dental pulp is richly supplied by a microcirculatory system comprising pre-capillary arterioles, capillaries and post-capillary venules. The arterioles enter through the apical foramen in a neurovascular collagen bundle, ascend towards the coronal region giving off numerous branches en route and terminate in a rich subodontoblastic capillary plexus. Pulp nociceptors are rarely activated by the throng of stimuli that teeth are exposed to in the oral environment during eating and drinking, unless the integrity of the protective hard casing surrounding the pulpo-dentine complex or the neurovascular bundle at the periapical region is compromised in pathological processes such as caries or trauma. Peripheral detection by pulp nociceptors which have their control centre (first-order neurons) located far away from the tooth in the trigeminal ganglia, the nociceptive signals are processed in the pars caudalis of the spinal trigeminal nucleus (second-order neurons) located in the medulla, and finally perceived by neurons that leave the thalamus and extend to the cerebral cortex in the central nervous system (CNS). Pulp inflammatory pain is predominantly due to the activation of unmyelinated C fibre nociceptors in the pulp proper. Pulp inflammation is often associated with a mixture

of inflammatory substances, including the presence of bacteria and their by-products, an influx of immune cells with activation of the cytokine network and inflammatory mediators which induces molecular changes in the nociceptive nerve fibres before pain is detected in the affected tooth – such as increased neuropeptide expression and sprouting of nociceptor nerve terminals. The low compliance environment in which the dental pulp is allocated enhances the complexity of its pathophysiology, explaining the unique responses of pulp tissue to pathologies like caries and periodontal diseases, as well as to additional injuries provoked by dental treatment such as mechanical and chemical irritation from cavity preparation and dental materials that could lead to neurogenic inflammation and consequently pulp necrosis and periapical disease.

MATERIALS

MATERIALS

2% Lignocaine with 1: 80,000 Adrenaline – (Lignox, Warren Pharmaceuticals, India)

Rubber dam (dental dam kit winged Coltene/Whaledent GmbH +Co.KG Germany)

K files size 15-25 (MANI, INC.Japan)

Endoprep – RC EDTA 15%, Cardamide peroxide 10% (Anabond Stedman Kanchipuram,Tamil nadu)

Paper points .06 taper (DIADENT Group International Korea)

Gutta percha points .06 taper (DIADENT Group International Korea)

24 gauge bevelled needle

Eppendorf tube

Phosphate-buffered saline PH 7.4 1X

Root ZX Mini Apex Locator (J Morita, Japan)

RaCe files (FKG Dentaire, Switzerland)

Endomate DT motor (NSK inc., Japan).

Saline (NS 500 ml, Sodium chloride 0.9%, Fresenius Kabi, India Pvt. Ltd)

3% sodium hypochlorite (Septodont Healthcare India Pvt. Ltd)

Ketorolac tromethamine (Ketorolac tromethamine Inj. IP 1 ml E.G.Pharmaceuticals, Solan (H.P) India)

Dexamethasone sodium phosphate (Dexalab Inj 2 ml, Laborate Pharmaceuticals, Sahib (H.P), India)

Enzyme-linked immunosorbent assay (ELISA) kit (Substance P ELISA kit Batch Number 0525422, Cayman Chemical, USA

Human IL-8 ELISA kit Batch Number 1804010, Shanghai, China).

METHODS

METHODS

After obtaining approval from the Ethics Committee of the institution. A sample size of 42 patients was calculated to be sufficient to detect clinical and biochemical data difference (alpha error of 0.05, power of 95% and effect size 0.7) (G power 3.1.9.2. software, Germany). All the patients reported to Department of Conservative Dentistry and Endodontics with a complaint of pain due to acute irreversible pulpitis from carious premolar and molar teeth. All the patients reported with moderate to severe pain of continuous or nocturnal, radiating or throbbing pain. All the teeth included in the study responded to the cold sensibility test with exaggerated pain with or without lingering manner. Excluded are restored ,root fractures, periapical pathology, variations in roots, pregnant women, mentally challenged, cardiac patients, patients taking medication 3 days prior.

Subjects were allotted to four different groups according to the irrigation done during root canal treatment. Allocation was done by block randomization with regards to pre-operative pain intensity (moderate or severe). The four irrigation groups were: saline (n=11), 3% sodium hypochlorite (n=11), ketorolac tromethamine (n=10), and dexamethasone(n=10)

Sample collection:

The levels of intensity pain for each patient pre-operatively and 24 hours post operatively were recorded using a 10 point visual analog scale. The patients expressed the intensity of pain by choosing a number using the following values: levels 0 – 3, mild pain, levels 4 – 7, moderate pain, and levels 8 – 10, severe pain.

Local anaesthesia (2% lignocaine with 1: 80,000 adrenaline - Lignox, Warren Pharmaceuticals, India) administration was given before root canal treatment. Each tooth was isolated with rubber dam during treatment. The pulpal blood samples (S1) was collected using a sterile paper points from the pulp chamber upon access gaining. The collected samples were stored in an Eppendorf tube and stored in -40˚C for further analysis.

Root canal treatment was done with working length estimation using a Root ZX Mini Apex Locator (J Morita, Japan) and RaCe files (FKG Dentaire, Switzerland) were used for root canal preparation according to the manufacturer's instructions with an Endomate DT motor (NSK inc., Japan). Depending on the patient allotment to the irrigation groups, saline (NS 500 ml, Sodium chloride 0.9%, Fresenius Kabi, India Pvt. Ltd), 3% sodium hypochlorite (Septodont Healthcare India Pvt. Ltd), ketorolac tromethamine (Ketorolac tromethamine Inj. IP 1 ml E.G.Pharmaceuticals, Solan (H.P) ,India) or dexamethasone sodium phosphate (Dexalab Inj 2 ml, Laborate Pharmaceuticals, Sahib (H.P), India) were used as irrigants during the root canal preparation procedures. In all cases, saline was used as the initial irrigant and this was followed by the sodium hypochlorite, ketorolac tromethamine or dexamethasone, according to the group allocation, as mid-rinses with up to 1ml only for each canal. Finally final irrigation for each canal was performed by saline with povidone iodine. Irrigation was performed with 24 gauge bevelled needle. The needle was inserted as far apically into the canal as possible but without any binding to the canal wall. Gentle force was used on the syringe to deliver the irrigant and the needle was moved up and down inside the canal to assist with irrigant flow and to ensure no binding of the needle to the canal wall. If any patients required additional local anaesthetic (either by

block or infiltration injection) or if they experienced pain during root canal treatment then this was recorded as intra-operative pain.

After root canal preparation apical patency was checked using a K file. A periapical blood sample (S2) was taken with a sterile paper point that was placed beyond the apical foramen immediately after root canal preparation. The paper points were left about 30 seconds and the collected sample was placed in the Eppendorf tube and stored in 40˚C for further analysis. The root canal fillings completed using gutta percha points with a zinc oxide eugenol sealer. After completing the occlusion was checked and relived. Analgesics were prescribed but the patients advised to take only need where there is not tolerant pain. The post operative pain levels and the analgesics taken were recorded after 24 and 48 hours by telephoning each patient.

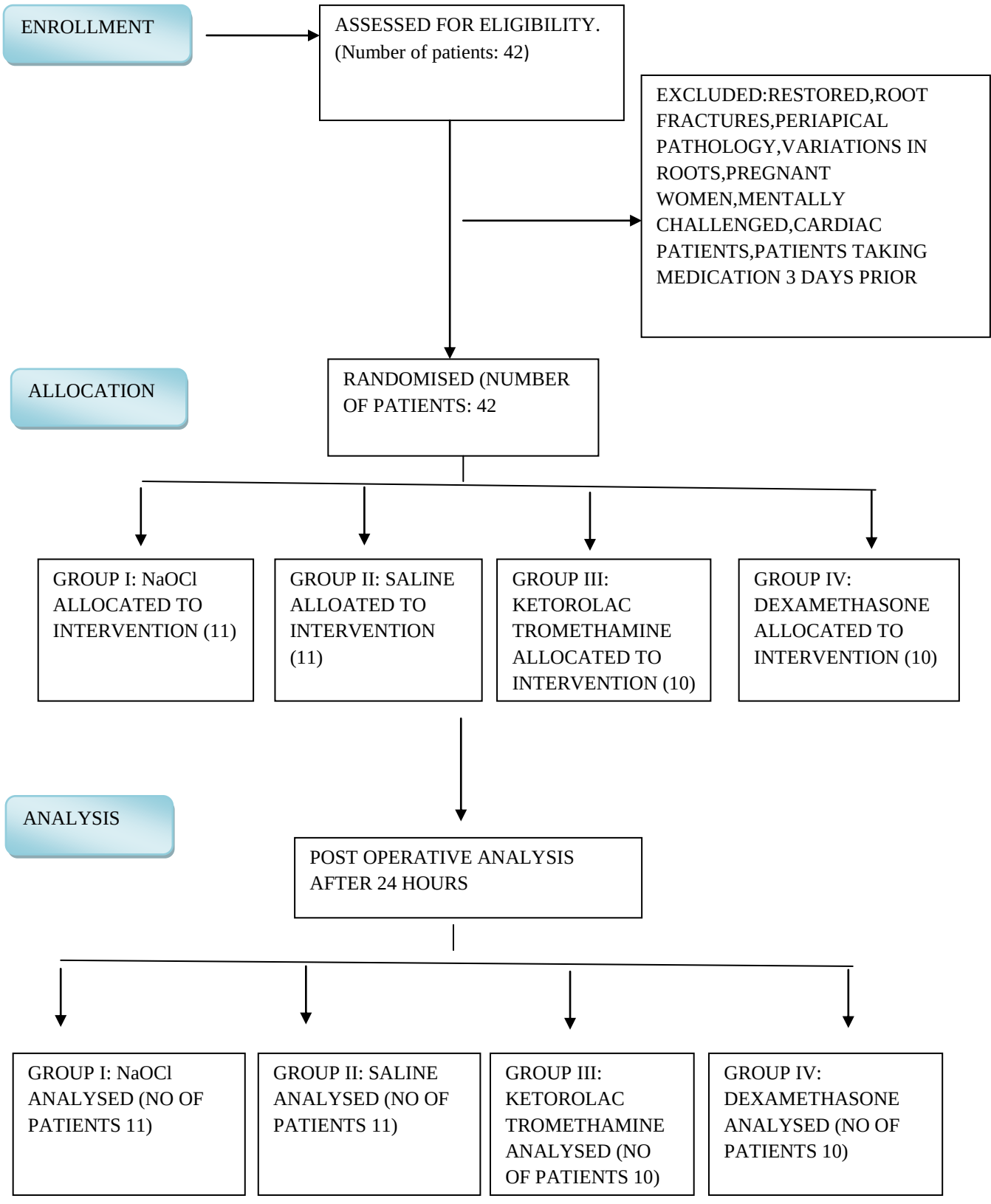
Biochemical analysis

The paper points were fractionated and diluted by using 600 µL (pH 7.4) phosphate-buffered saline. Then the samples were vortexed and centrifuged at 10,000 rpm for 5 minutes. Quantification of substance P and IL-8 was completed according to the manufacturer's instructions using an enzyme-linked immunosorbent assay (ELISA) kit (Substance P ELISA kit Batch Number 0525422, Cayman Chemical, USA and Human IL-8 ELISA kit Batch Number 1804010, Shanghai, China). The lower limits of detection for substance P and IL-8 ELISA kits were3.9 pg/ml and 1.07 pg/ml respectively. The absorbency of each sample was read at 420-450 nm wavelengths in a microplate reader (SpectroMaxPlus 384, USA). A standard curve was created using the standard concentrations of substance P and IL-8. The concentrations of substance P and IL-8 for each sample were calculated using the standard curve.

Statistical analysis

Statistical analysis was performed using IBM SPSS software version 23 (IBM Corp., USA). Categorical values of the pre-operative pain intensity, intra-operative pain occurrence, analgesics requirement and post-operative pain intensity were compared using the Chi-square test. Pearsons correlation coefficients were calculated between the pain scores and substance P values. Normality of pre-operative pain, post-operative pain scores, substance P and IL-8 data were checked by the Shapiro-Wilk test. The data was skewed and deviated from normal distribution- therefore the comparison of these values for the different irrigation groups were done by non-parametric Wilcoxon signed rank and Kruskal Wallis tests. The level of significance was set at 5%.

CONSORT FLOW CHART 2010:



FIGURES

FIGURES

FIGURE 1: ARMAMENTARIUM

FIGURE 2: DIFFERENT IRRIGATING SOLUTIONS

FIGURE 3: PHOSPHATE BUFFERED SALINE AND EPPENDORF TUBE

FIGURE 4: RUBBER DAM KIT

FIGURE 5: SUBSTANCE P ELISA KIT

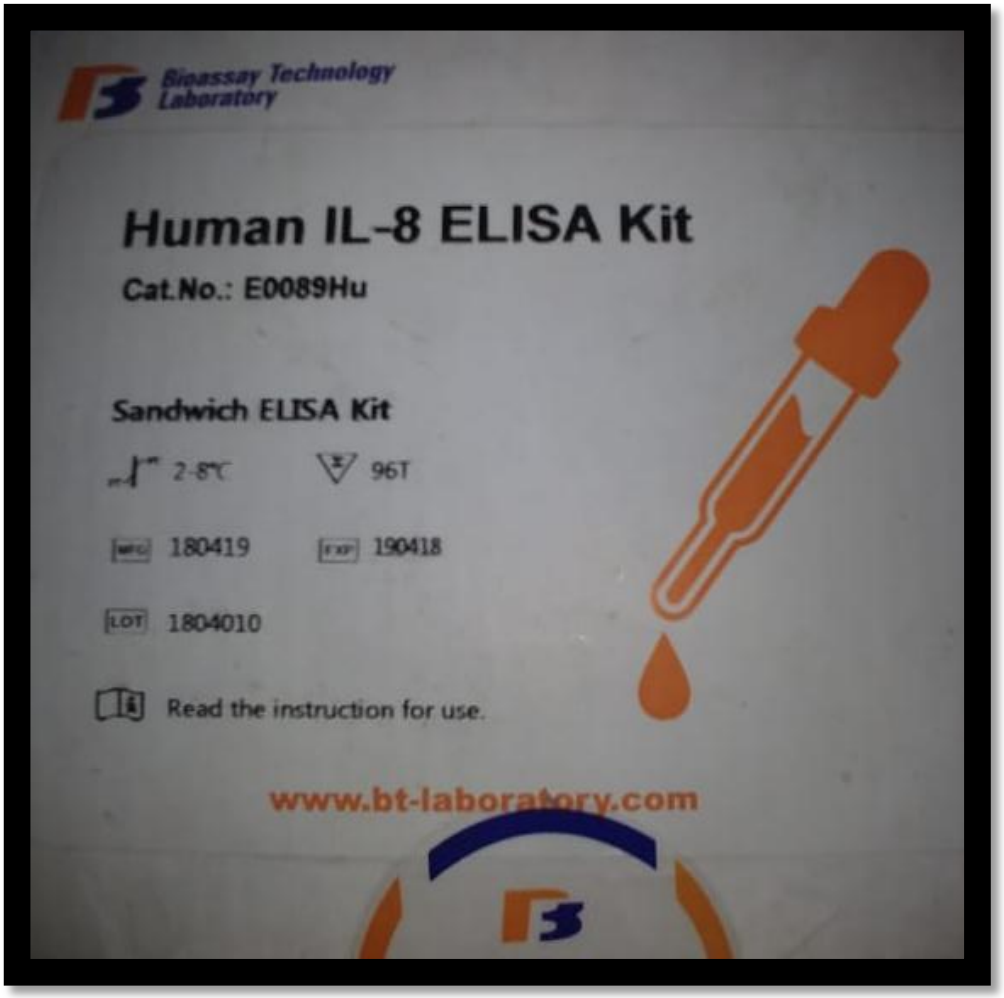


FIGURE 6: IL-8 KIT

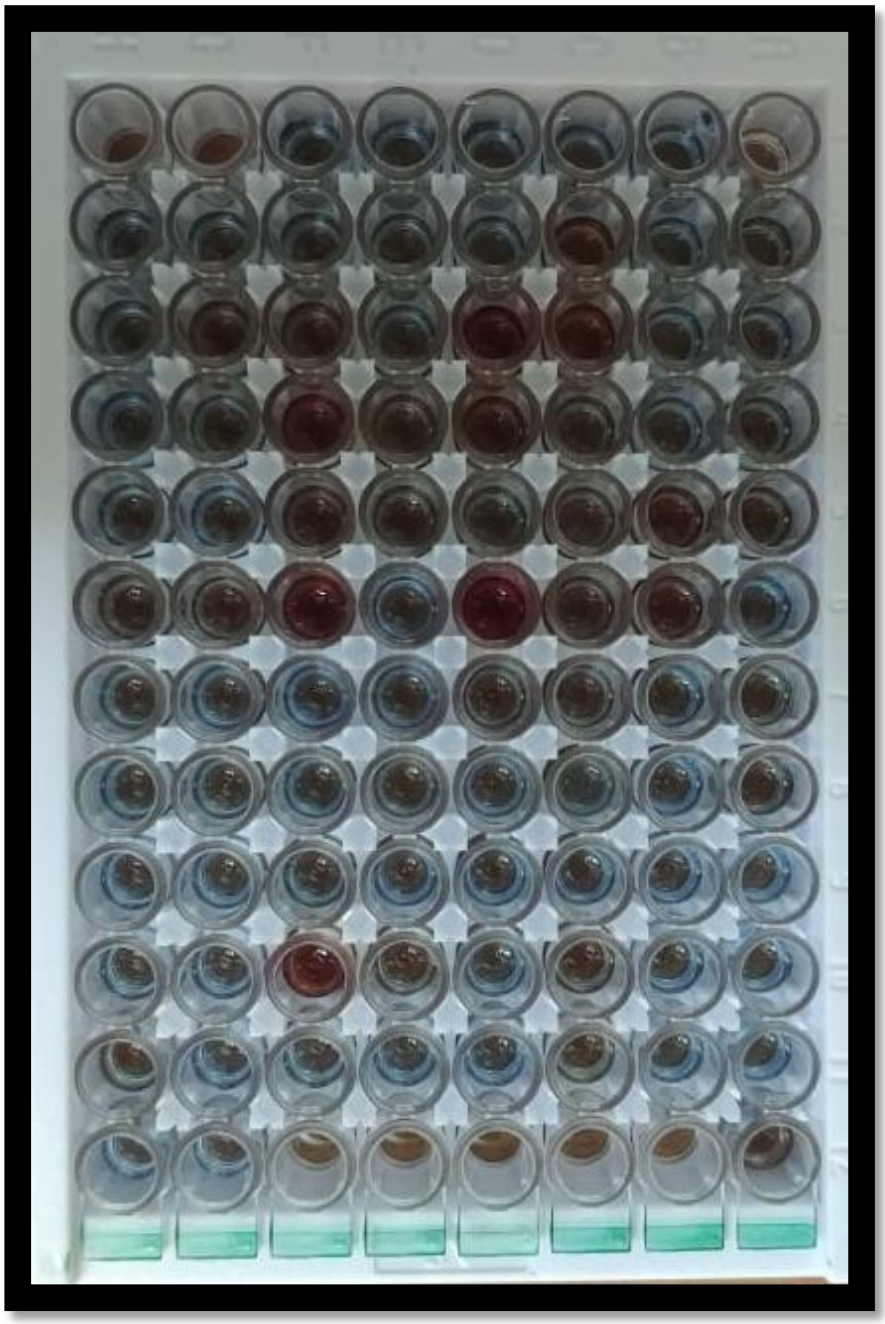


FIGURE 7: SUBSTANCE P KIT WITH 96 WELLS

FIGURE 8: SUBSTANCE P 96 WELLS AFTER SAMPLE PREPARATION

FIGURE 9: SUSTANCE P 96 WELLS AFTER FINAL RESULT

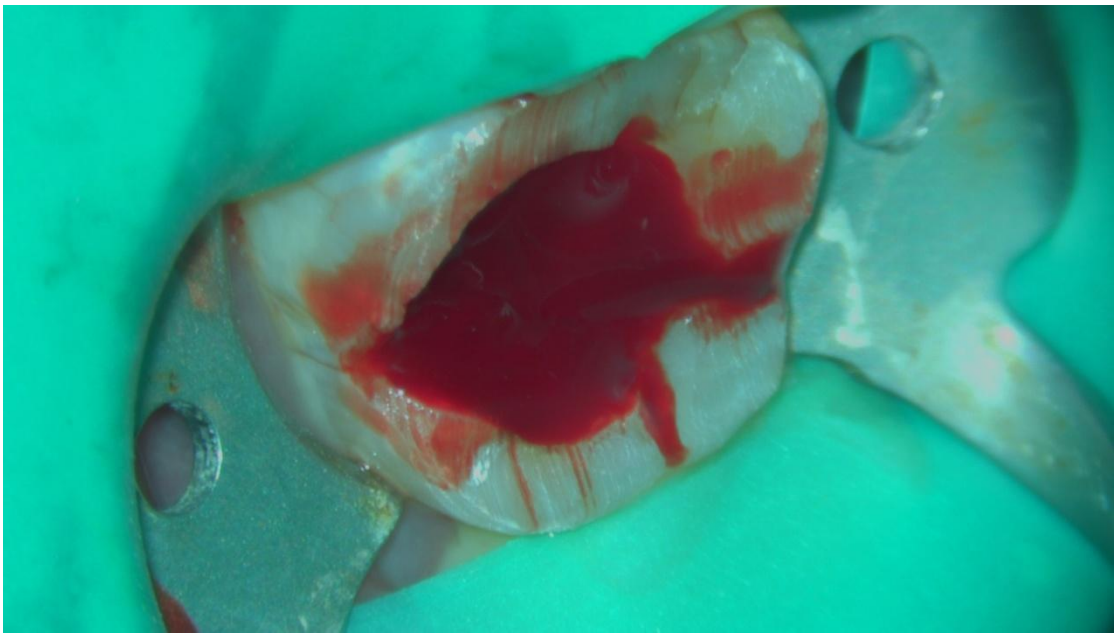


FIGURE 10: POOLING OF BLOOD AFTER ACCESS OPENING

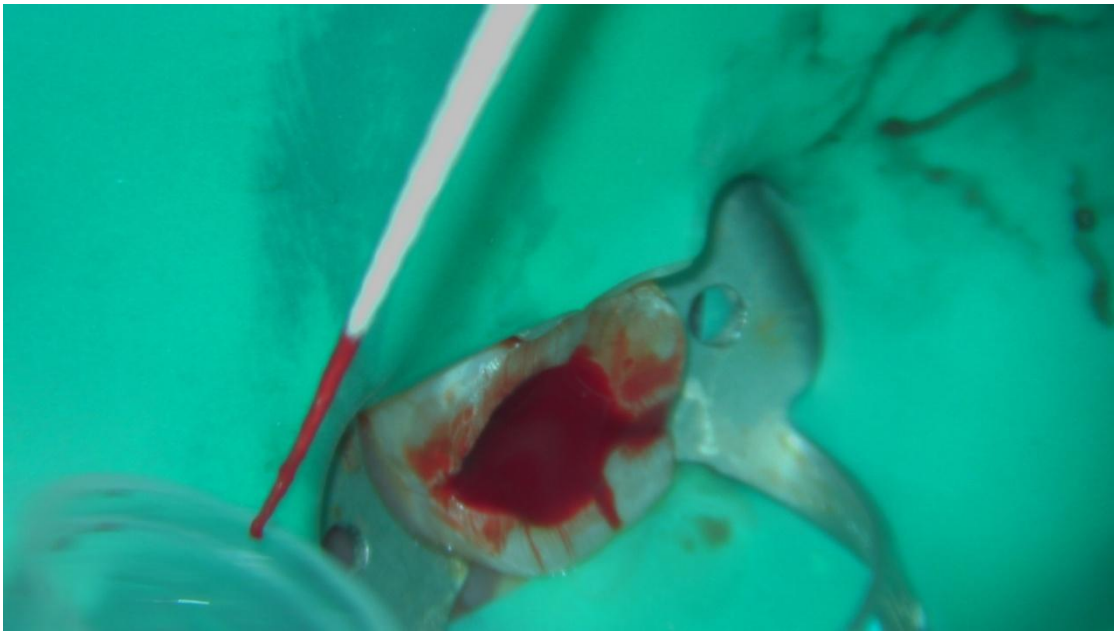


FIGURE 11: SAMPLE TAKEN WITH PAPER POINTS IMMEDIATE AFTER ACCESS OPEINING

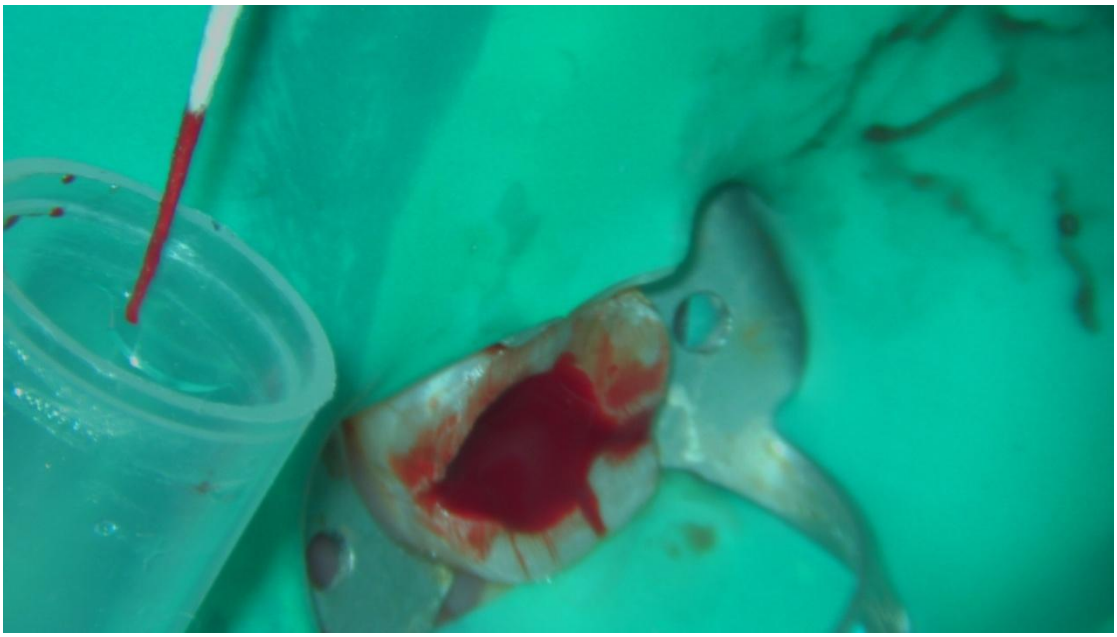


FIGURE 12: SAMPLES ARE STORED IN EPPENDORF TUBE WITH PHOSPHATE BUFFERED SALINE

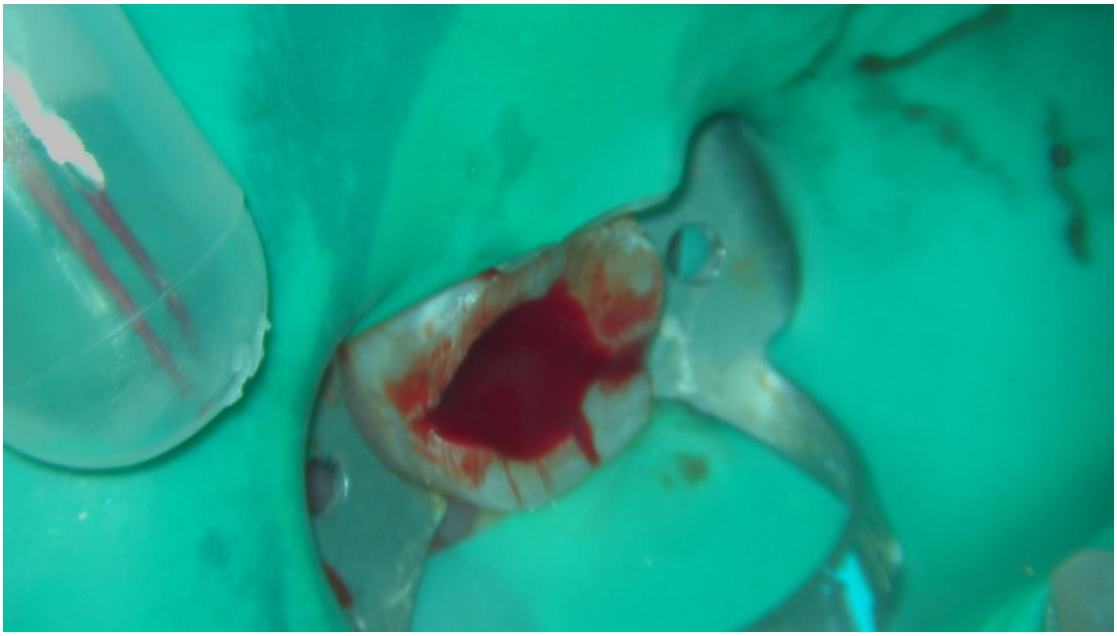


FIGURE 13: SAMPLES STORED IN EPPENDORF TUBE

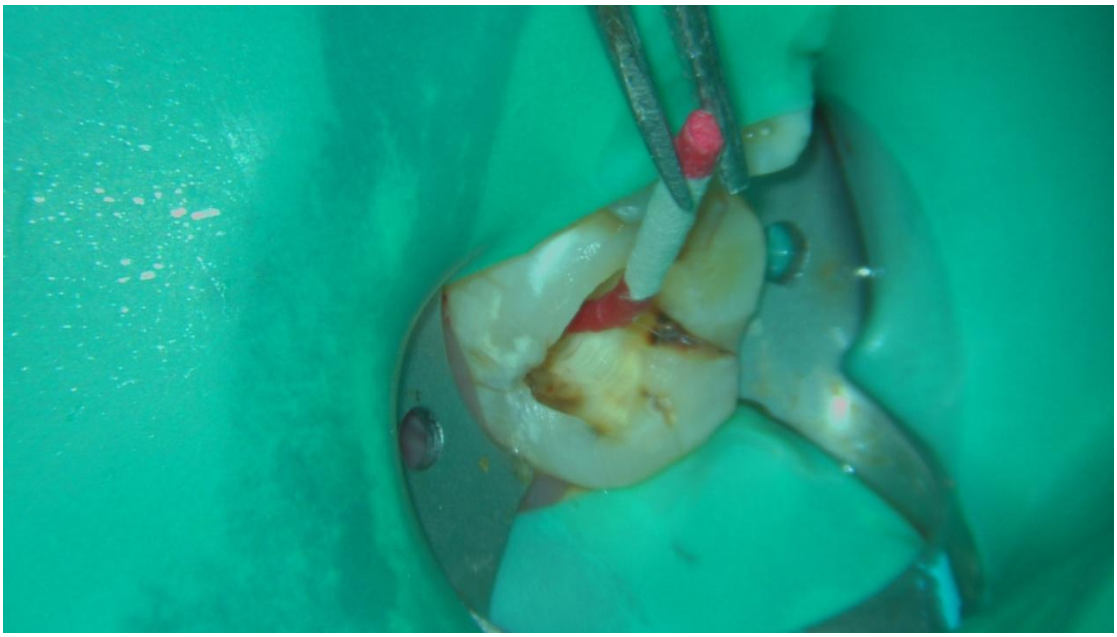


FIGURE 14: SAMPLES TAKES AFTER BIOMECHANICAL PREPARATION

RESULTS

RESULTS

Graph 1 illustrates gender frequency distribution of forty two patients with 20 males (48%) and 22 females (52%) aged between 15 to 65 years were participated in this study. Table 1 shows the mean preoperative pain score was 7.09 (standard deviation 1.33). Table 2 shows fourteen patients (33.3 %) required analgesics post-operatively for pain control and 28 patients (66.7 %) did not require analgesics. Table 3 shows post-operative pain occurrence was 40.5 % (17 patients). Table 4 shows there was a significant difference ($p < 0.05$) between the pre-operative and 24 hrs post-operative pain scores with 24 hrs post-operative pain being significantly less (Wilcoxon signed Rank tests). Graph 2 illustrates a significant difference ($p < 0.05$) between 24 hrs and 48 hrs post-operative pain scores (Wilcoxon signed Rank tests) was observed for all patients with the mean 48 hrs pain score being zero. Graph 3 illustrates female patients had significantly higher ($p < 0.05$) 24 hrs post-operative pain scores (mean score – 1.86) and incidence of post-operative pain (59.1 %) (Chi-squared test). Table 5 and 6 shows there is no significant association was seen between gender and S1, S2 substance P, IL-8 expression values. It was noticed that, males had elevated values of substance P and IL-8 except for S2 IL-8 values. Table 7 shows that lower molar teeth (12 of 17 patients) had significantly ($p < 0.05$) higher pre-operative pain scores (7.94) compared to other teeth (Chi squared test). Lower molar teeth also had an increased incidence (7 patients) of post-operative pain compared to other teeth. Table 8 shows that in all four irrigant groups, there were significant differences ($p < 0.05$) between the pre-operative pain scores and the 24hrs post-operative pain scores (Wilcoxon signed Rank tests). A significant difference ($p < 0.05$) between the 24 hrs and 48 hrs post-operative pain scores was observed only with sodium hypochlorite group. Table

8 shows that the mean post-operative 24 hrs (mean score – 2) and 48 hrs pain scores (mean score 0.45) was highest for sodium hypochlorite compared to the other irrigant groups. Only one male patient from the sodium hypochlorite irrigation group reported pain at 48 hrs post-operatively. Table 9 shows that among the four irrigant groups, dexamethsone had the least number of patients (2 patients) requiring analgesics for post-operative pain and also the lowest post-operative pain incidence. The sodium hypochlorite group had the highest number of patients (6 patients) requiring post-operative analgesics and 7 patients reported post-operative pain. Table 10 shows that females (11 patients) had a higher incidence of intra-operative pain in this study. Table 11 shows that a significantly ($p < 0.05$) higher number of lower molar teeth (13 of total 17 patients) had intra-operative pain compared to other tooth types (Chi square test). Graph 4 shows that the mean S1 values of substance P (105.92 pg/ml) and IL-8 (17.52 pg/ml) were higher for lower molar tooth than other tooth types. Table 12 shows that there was a significantly ($p< 0.5$) increased mean S1 substance P value (112.41 pg/ml) when patients experienced intra-operative pain. Table 13 and 14 shows that Pre-operative and post-operative pain values did not show significant associations with substance P and IL-8 values (Kruskal Wallis test). Table 14 shows that the mean IL-8 S2 value was highest for patients with moderate intensity 24 hrs post-operative pain and was least for patients with no pain. Table 14 shows that with substance P, the highest values were observed with patients having no 24 hrs post-operative symptoms. Table 15 shows that the difference between S1 and S2 sample values for substance P and IL-8 between the four different irrigant groups was not significant (Wilcoxon signed Rank tests). The sodium hypochlorite group had a higher mean expression of substance P and IL-8 values in the S2 samples than the other irrigant groups. Lower expressions of substance P and IL-8 in S2 sample values were observed with the

saline and ketorol irrigation groups. Graph 5 illustrates that in patients requiring analgesics for post-operative pain control, there was an increased expression of IL-8 values in the S2 samples compared to patients who did not require analgesics.

TABLES AND GRAPHS

TABLES AND GRAPHS

GRAPH 1: FREQUENCY DISTRIBUTION OF GENDER

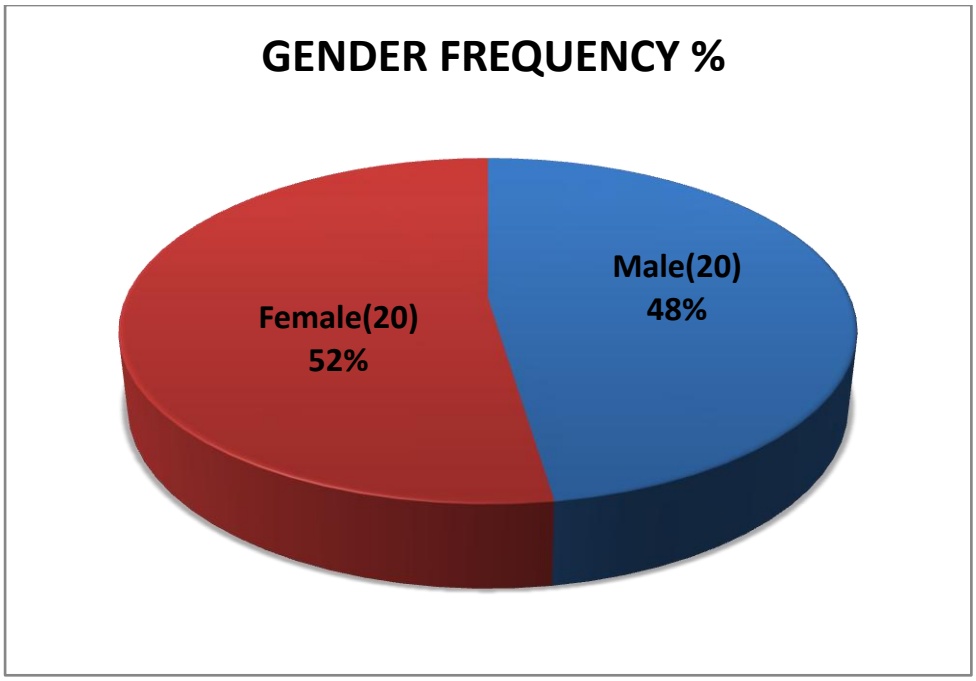


TABLE 1: COMPARISION OF PREOPERATIVE PAIN SCALE AND POST OPERATIVE 24 HOURS PAIN SCALE

Duration	No. of observations	Mean (SD)	Range	P value[W]
Pre-op pain scale	42	7.09 (1.33)	5 -10	<0.0001
Post op 24 hours pain scale	42	1.31 (1.75)	0 – 6	

TABLE 2: COMPARISION OF ANALGESIS REQURIED AND ANALGESIS NOT REQUIRED

POST OP ANALGESIS	Frequency (%)
ANALGESIS REQUIRED	14(33.3%)
ANALGESIS NOT REQUIRED	28(66.7%)

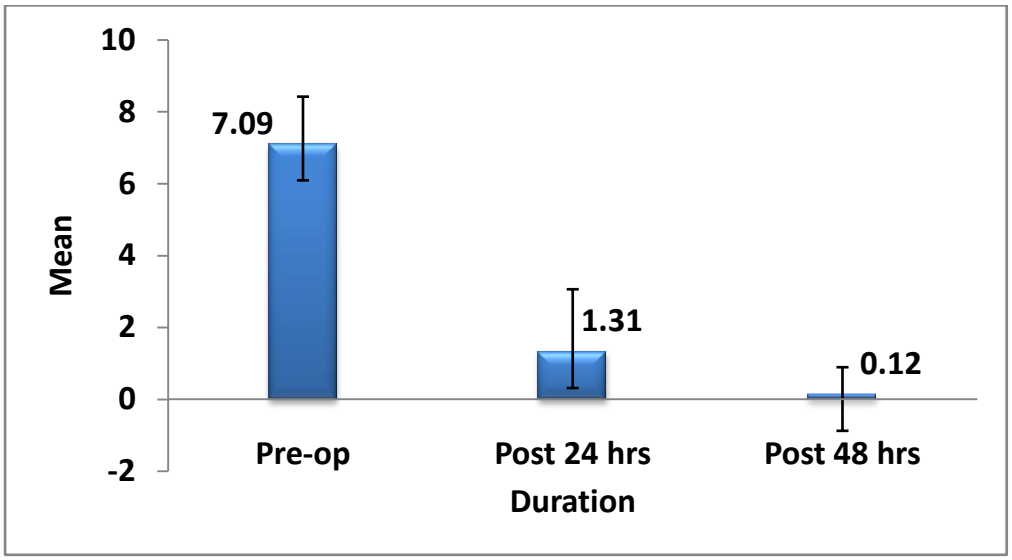
TABLE 3: FREQUENCY DISTRIBUTION OF POST OPERATIVE PAIN

Post –op pain	Frequency (%)
No	25 (59.52)
Yes	17 (40.48)
Total	42 (100)

TABLE 4: COMPARISION OF POST OPERATIVE PAIN SCALE 24 HOURS AND POST OPERATIVE PAIN SCALE 48 HOURS

Duration	No. of observations	Mean (SD)	Range	P value[w]
Post op 24 hours pain scale	42	1.31 (1.75)	0 – 6	<0.0001
Post op 48 hours pain scale	42	0.12 (0.77)	0 - 5	

GRAPH 2: BAR CHART FOR PAIN SCORES AT DIFFERENT TIME POINTS



GRAPH 3: BAR CHART SHOWS PREOPERATIVE PAIN SCALE AND POST OPERATIVE PAIN SCALE BETWEEN GENDERS

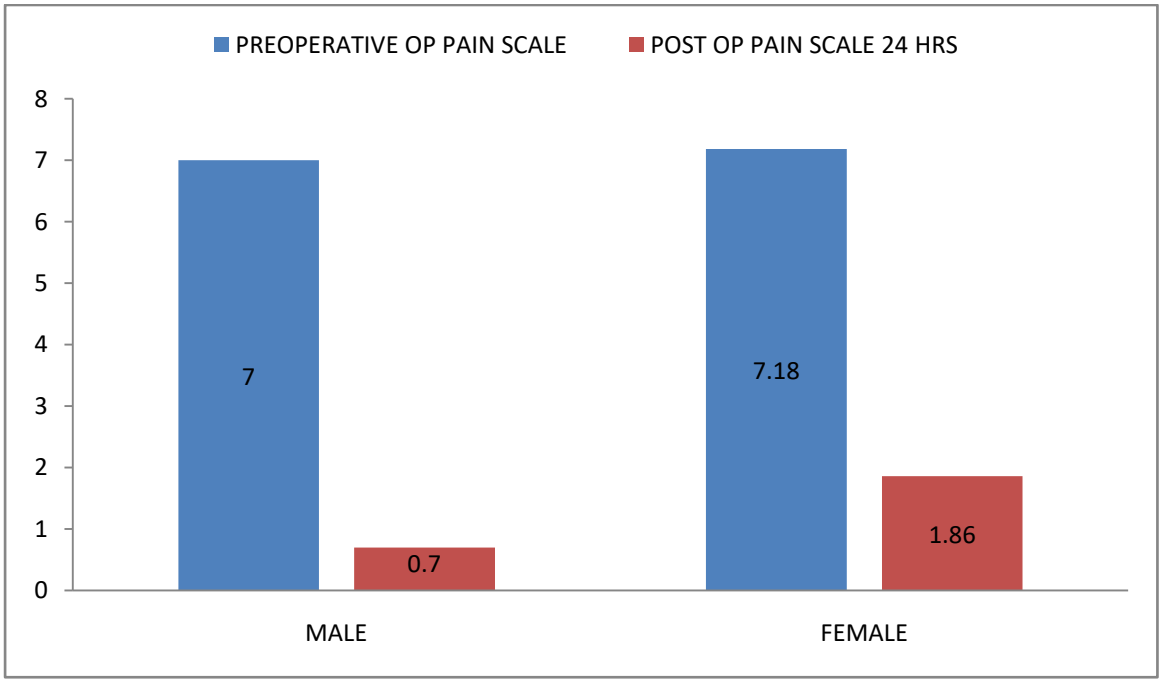


TABLE 5: COMPARISON OF S1AND S2 SUB P BETWEEN GENDERS

Gender	No. of observations	Mean (SD)	Range	P value[M]
Male SI S2	19	 100.73(150.26) 63.52(66.88)	3.9 – 500	For S1 0.6896 (NS)
Female S1 S2	22	 65.69 (71.09) 51.13(46.84)	7.1 - 250	For S2 0.8414(NS)

TABLE 6 COMPARISON OF S1AND S2 IL8 BETWEEN GENDERS

Gender	No. of observations	Mean (SD)	Range	P value[T]
Male SI S2	19	 17.57 (8.99) 10.89(9.51)	0 – 25	For S1 0.1485 (NS)
Female S1 S2	22	 13.13 (10.12) 13.81(10.64)	0 - 25	For S2 0.3689(NS)

TABLE 7: COMPARISON OF PREOPERATIVE PAIN INTENSITY AND TOOTH QUADRANT

Tooth Quadrant	Pre-op Pain intensity		Total N (%)	P value[C]
	Moderate N (%)	Severe N (%)		
Lower Molar	5 (21.74)	12 (63.16)	17 (40.48)	0.031 (S)
Lower Premolar	2 (8.70)	2 (10.53)	4 (9.52)	
Upper Molar	11 (47.83)	4 (21.05)	15 (35.71)	
Upper Premolar	5 (21.74)	1 (5.26)	6 (14.29)	
Total	23 (100)	19 (100)	42 (100)	

TABLE 8: DISTRIBUTION OF PAIN SCORES IN DIFFERENT IRRIGATION GROUPS

IRRIGATION GROUP	PRE OP PAIN SCALE mean	P value	POST OP PAIN SCALE 24 HRS mean	P value	POST OP PAIN SCALE 48 HRS mean	P value
SALINE	6.73	0.4884(NS)	.91	0.3956(NS)	.00	0.9781(NS)
SODIUM HYPOCHLORITE	7.18		2.00		.45	
KETORAL	6.90		1.40		.00	
DEXAMETHASONE	7.60		.90		.00	

TABLE 9: COMPARISON OF IRRIGATION GROUPS AND POST OPERATIVE ANALGESICS

Irrigation group	Post –op Analgesics		Total N (%)	P value [F]
	Analgesics not required N (%)	Analgesics required N (%)		
Dexamethasone	8 (28.57)	2 (14.29)	10 (23.81)	0.404 (NS)
Ketorol	7 (25)	3 (21.43)	10 (23.81)	
Saline	8 (28.57)	3 (21.43)	11 (26.19)	
Sodium hypocholorite	5 (17.86)	6 (42.86)	11 (26.19)	
Total	28 (100)	14 (100)	42 (100)	

TABLE 10: COMPARISON OF HOT TOOTH AND GENDER

Gender	Hot tooth		Total N (%)	P value[C]
	No N (%)	Yes N (%)		
Male	13 (54.17)	7 (38.89)	20 (47.62)	0.327 (NS)
Female	11 (45.83)	11 (61.11)	22 (52.38)	
Total	24 (100)	18 (100)	42 (100)	

TABLE 11: COMPARISON OF HOT TOOTH AND TOOTH QUADRANT

Tooth Quadrant	Hot tooth		Total N (%)	P value[F]
	No N (%)	Yes N (%)		
Lower Molar	4 (16.67)	13 (72.22)	17 (40.48)	0.001 (S)
Lower Premolar	2 (8.33)	2 (11.11)	4 (9.52)	
Upper Molar	13 (54.17)	2 (11.11)	15 (35.71)	
Upper Premolar	5 (20.83)	1 (5.56)	6 (14.29)	
Total	24 (100)	18 (100)	42 (100)	

GRAPH 4: BAR GRAPH REPRENTATION OF MEAN SUBSTANCE P (SI) AND IL-8 (S1) Pg/ml VALUES FROM DIFFERENT TOOTH TYPES

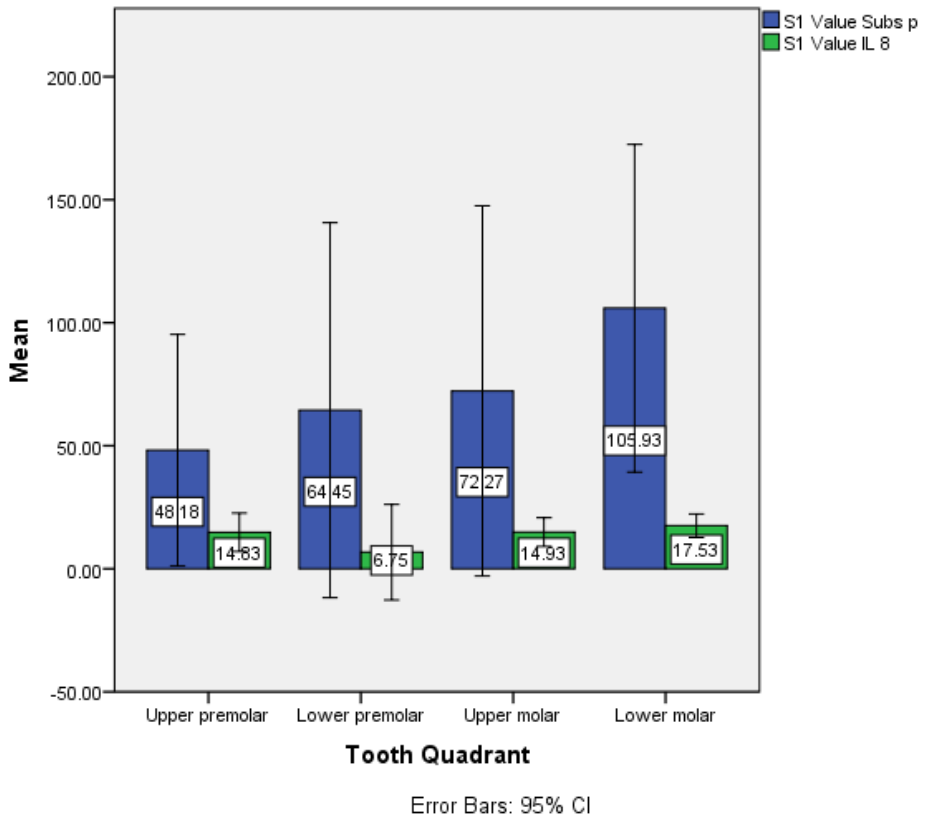


TABLE 12:INTRAOPERATIVE PAIN MEAN S1 VALUES OF SUBSTANCE P AND IL-8 EXPRESSION USING KRUSKAL WALLIS TEST

INTRAOPERATIVE PAIN	**S1 Value subsP**	***P value***	**S1 value IL 8**	***P* value**
Yes mean N Std.Deviation	112.4133 18 122.39167	0.0206(NS)	16.3333 18 9.31791	0.406(NS)
No mean N Std.Deviation	58.0848 23 104.59231		14.3034 23 10.21334	

TABLE 13: MEAN PREOPERATIV EPAIN SCORE COMPARISON WITH SUBSTANCE P(S1) AND IL-8 (S1) VALUE (pg/ml)

PRE OPERATIVE PAIN VALUE	S1 Value subsP	S1 value IL 8
MODERATE PAIN mean N Std.Deviation	82.0314(0.162) 22 141.37736	15.1364(0.968) 22 9.82851
SEVERE PAIN mean N Std.Deviation	81.8263(0.162) 19 76.51801	15.1364 19 9.95458

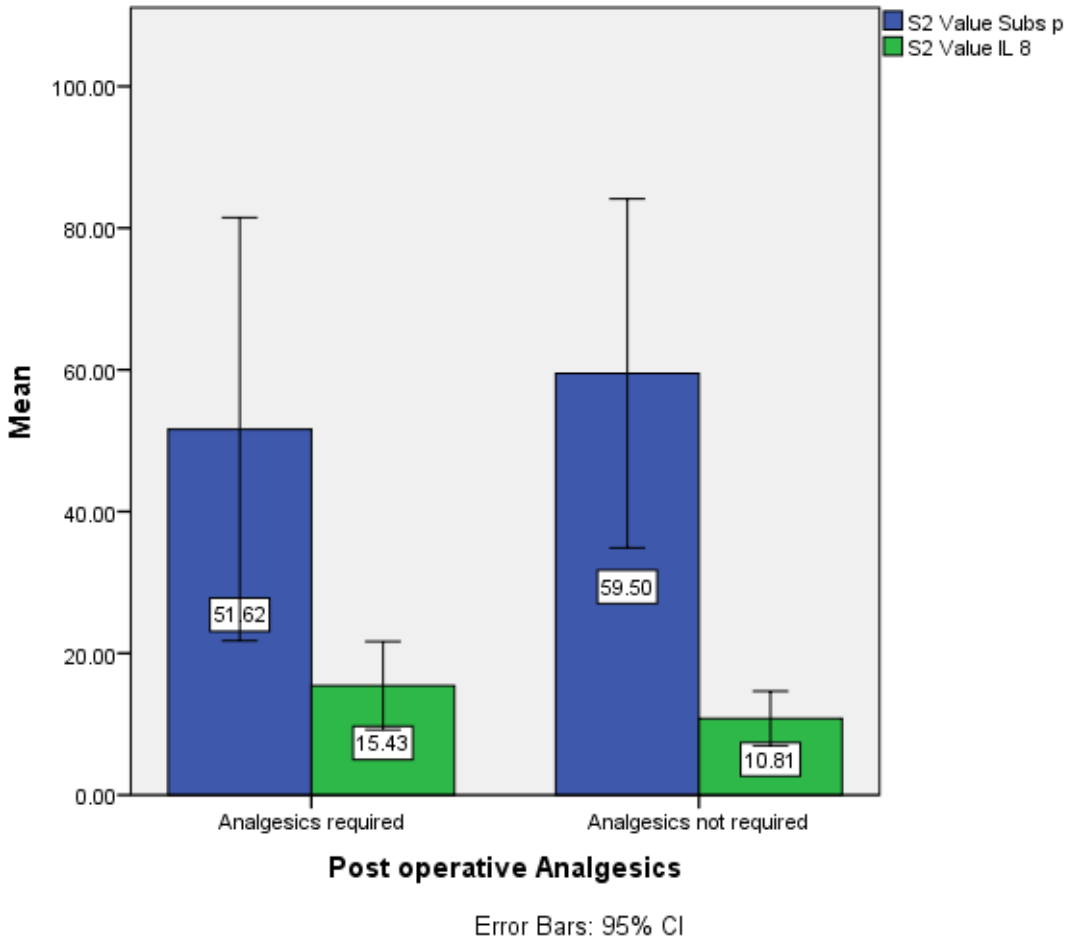
TABLE 14: MEAN POST OPERATIVE 24 HOURS PAIN SCORE COMPARISON WITH SUBSTANCE P(S2) AND IL-8(S2) VALUE (pg/ml)

POST PERATIVE PAIN 24 HRS	S2 Value subsP	S2 value IL 8
NO PAIN mean N Std.Deviation	63.6400(0.743) 24 61.64954	11.4783(0.578) 23 9.51969
MODERATE PAIN mean N Std.Deviation	48.1767(0.743) 12 50.69512	12.5833(0.578) 12 11.54799
SEVERE PAIN mean N Std.Deviation	45.3140(0.743) 5 49.53294	16.4000(0.57) 5 10.23719

TABLE15: COMPARISON OF S1 AND S2 SUBSTANCE P AND S1 AND S2 IL-8 VALUES

IRRIGATION GROUP	S1 Value subsP	Pvalue	S2 Value subsP	*P* value	S1 value IL 8	P value	S2 value IL 8	*P* value
SALINE SODIUM HYPOCHLORITE KETORAL DEXAMETHASONE	83.45[.248] 125.39[.500] 54.69[.203] 64.06[.672]	0.9979(NS)	43.68[.248] 67.97[.500] 56.64[.203] 60.55[.672]	0.6789(NS)	16.27[.206] 16.90[.462] 11.00[.672] 12.40[.721]	0.9672(NS)	11.27[.206] 15.44[.462] 11.00[.672] 12.40[.721	0.7798()NS

GRAPH 5: BAR GRAPH REPRESENTS THE COMPARISON OF MEAN SUBSTANCE P AND IL-8 (S2) pg/ml VALUES WITH POST ANALGESICS REQUIRED



DISCUSSION

DISCUSSION

The main aim of this study was to assess the influences of ketorolac tromethamine and dexamethasone used as root canal irrigant in root canal treatment for teeth with acute irreversible pulpitis and on substance P and IL-8 expression in the pulp and periapical tissues. In this study the results reveals that ketorolac tromethamine had better control in the expression of substance P and IL-8 compared to dexamethasone. In the 24 hours post operative pain seventeen patients (40.5%) reported pain. In a systematic review the 24 hours post treatment pain is lower (28%) and the observation is higher in our study[18].

In another study for single visit root canal treatment done in teeth with pulp status classified as normal, irreversible pulpitis and necrotic, here women recorded higher percentage (42%) of slight pain compared to males (26%) [19]. In a prospective study resulted that preoperative pain was a good predictor of incidence of pain, tooth type, age of the patient, not of the intensity of post endodontic pain. In our study fourteen patients (33.3%) required analgesics[20]. All the patients took analgesics for only one dose that is 24 hours post operatively which was able to control pain. Use of analgesics in this study has shown to be effective in controlling post operative pain. And in 48 hours post operative pain score was reduced to zero. In this study females had significantly higher 24 hours post operative pain score and pain incidence. In previous studies also reported that increased incidence of post operative pain in females[20, 21]. In the current study in spite of these findings no significant difference between substance P and IL-8 expressions between both the genders. Khan[27] et al found that significantly high levels of mechanical allodynia, as reduced mechanical

pain thresholds in women with irreversible pulpitis. In our study the observations significantly females (10 patients 45.5%) reported with mild pain compared to males (2 patients 10%) post operatively.

In this study lower molar tooth had significantly increased preoperative pain intensity and higher occurrence of post operative pain. In previous studies significantly higher percentage of slight pain in the mandible found due to nerve block injections because they are more technically difficult compared to other injections[19]. Another study reported that the mandible has a thicker cortical plate other than the maxilla this could be the reason for more intense pain in mandibular arch[20].

Current results shows that mean pre operative substance P and IL-8(S1) values were higher for lower molar teeth compared to other teeth , this could explain that significantly increased incidence of pre operative pain intensity in teeth. In this study sodium hypochlorite irrigation group had higher mean post operative pain score at 24 and 48 hours compared to other irrigation groups. It was also observed that sodium hypochlorite group had higher mean expression substance P and IL-8 values (S2).In previous studies shows that IL-8 is produced from several pulpal cells, and odontoblast cells exhibits low level IL-8 expression that significantly increases the pathogen associated molecular pattern stimulation[16]. The increased IL-8 expression correlated with increased polymorphonuclear netrophls within the pulp because IL-8 induces neutrophil chemotaxis and release of degradation enzymes[16]. And this studies correlates IL-8 is a primary regulatory molecule in the acute inflammatory response and levels may perpetuate and exacerbate the acute inflammatory response in irreversible pulpitis[16].

In our study elevated expression of inflammatory mediators was associated with higher post operative pain scores for sodium hypochlorite group. In previous studies using two different concentrations of sodium hypochlorite as irrigant for single visit root canal treatment for mandibular molar teeth with irreversible pulpitis and there is no history of spontaneous pain and the post operative pain occurrence was highest during first 48 hours post operatively in both the irrigation groups[23]. However it was also observed that increased concentration of sodium hypochlorite had significantly less post operative pain occurrence[23]. In our study only patients with pre treatment pain of moderate to severe intensity and sodium hypochlorite irrigation was not able to control the post operative pain occurrence as effectively as other irrigation solutions. Lower level expression of the inflammatory mediators was found with saline and ketorolac tromethamine irrigation groups. Earlier study in the department ketorolac tromethamine with anti inflammatory properties was to be able to control the expression of substance P. In the saline group because as an inert material its ability to compare with other active irrigants with regard to its ability to control the expression of inflammatory mediators. Dexamethasone irrigation was able to achieve the least post operative pain score and requirement of analgesics. In spite of the findings in this study, of not having the least expression of substance P and IL-8 values in S2 samples. In a study by Glassman used dexamethasone as analgesics (oral usage) [24]. In another study dexamethasone is used as intra canal medicament for the management of necrotic and inflamed pulps in post operative pain during root canal treatment[26]. Only patients who had not taken preoperative analgesics in previous seven days prior to treatment were included in this study. In this study intra-operative pain incidence of 42.9 % associated with root canal treatment. Patients with intra-operative pain in this study had significantly increased expression of substance P. In previous

studies increases in neuropeptides have been shown in human pulp infected by caries,sprouting causes an increase in the density of innervation of the inflamed tissue, contributing to increased pain sensitivity in chronic pulpal inflammation[7].

S1 values of substance P and IL-8 did not show significant association with pre-operative pain intensity, this could be because in this study only moderate and severe intensity of pain patients were included in this study. It was also observed in this study, that patients with increased post-operative pain intensity and also who required analgesics for post-operative pain management had higher expression of IL-8 (S2 values). Higher levels of IL-8 role in pulpitis have been extensively reviewed and this molecule has been associated with PMNs. In this study it was seen, that substance P did not have association with post-operative pain occurrence or analgesics requirement.

SUMMARY

SUMMARY

The purpose of the study was to evaluate the effect of anti inflammatory irrigants on inflammatory mediators in root canal treatment in symptomatic vital teeth.

In this invivo study 42 patients are selected with symptomatic with irreversible pulpitis with complete root development and determined by both visual and radiograph. The teeth were equally divided into 4 groups. First the teeth was anesthetized by 2% lignocaine and the root canal procedure carried out. All teeth were prepared for 10 minutes and the canals were irrigated with different irrigating solution ,the teeth is isolated with rubber dam .The patients pre operative and post operative pain evaluated by visual analog scale. and the periapical tissue fluid samples were taken by paper points and stored in eppendorf tube, snap frozen in liquid nitrogen and kept at -70◦C until use. The substance P and IL-8 were determined by ELISA KIT (high sensitivity). The values were subjected to statistical analysis, Wilcoxon signed Rank tests and chi square test.

CONCLUSION

CONCLUSION

Root canal treatment in teeth with irreversible pulpitis is more painful. Within the limitations of this study we concluded that,

Post operative pain incidence following root canal treatment in symptomatic teeth is high. This pain occurrence was managed by analgesics within 48 hrs. Dexamthasone irrigation was more effective in control of post-operative symptoms in acute irreversible pulpitis. Females are more prone to post-operative pain occurrence. Il-8 expression was elevated in patients with increased post-operative pain intensity and requiring analgesics for pain control. Substance P level was significantly increased in patients with intra-operative pain. Lower molar teeth had higher incidence of pre-operative pain intensity, intra-operative pain and post operative pain occurrence; and also lower teeth had higher levels of substance P and IL-8 expression.

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REFERENCES

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ANNEXURES

ANNEXURE-I

The Diocese of Madurai - Ramnad

C.S.I. College of Dental Sciences and Research

129, East Veli Street, Madurai - 625 001. Tamilnadu, India.

Ph : 0452 - 2321708, 2336604 Fax : 2336605

Email ID : csidental@gmail.com Website : www.csidentalcollege.org

ETHICAL COMMITTEE

Title of the work : Effects of anti-inflammatory irrigants on inflammatory mediators in root canal treatment of symptomatic vital teeth. An In-Vitro study

Principal investigator :Dr.J. Evangelin, II MDS

Department: Conservative Dentistry & Endodontics **CSICDSR/IEC/0038/2017**

The request for an approval from the Institutional Ethical Committee (IEC) for the above mentioned study, submitted by the Principal investigator is considered in the IEC meeting held on 31.10.2017 at CSI College of Dental Sciences and Research, Madurai. The members of the committee are unanimously pleased to approve the proposed work mentioned above and is '**Advised to proceed with the study**'

The Principal investigator and their team are directed to adhere the guidelines given below:

1. You should get informed consent from the patients/participants and maintain confidentiality.
2. You should carry out the work without detrimental to regular activities as well as without extra expenditure to the Institution.
3. You should inform the IEC in case of any change of study procedure, site and investigation or guide.
4. You should not deviate from the area of work for which you have applied for ethical clearance.
5. You should inform the IEC immediately in case of any adverse events or serious adverse reactions. You should abide to the rules and regulations of the institution.
6. You should complete the work within the specific period and if any extension of time is required, you should apply for the permission again and do the work.
7. You should submit the summary of the work to the ethical committee on completion of the work.
8. You should not claim funds from the institution while doing the work or on completion.
9. You should understand that the members of IEC have the right to monitor the work with prior intimation.
10. Your work should be carried out under the direct supervision of your Guide/Professor.

Dr.A.Charles MS MCh

President

Dr.N.Gururaj MDS

Secretary

ANNEXURE-II

URKUND

Urkund Analysis Result

Analysed Document:	THESIS DOC.docx (D46474083)
Submitted:	1/7/2019 5:16:00 AM
Submitted By:	jevangelin90@gmail.com
Significance:	3 %

Sources included in the report:

FINAL THESIS 111.docx (D46339962)
http://www.aetna.com/cpb/medical/data/100_199/0113.html
https://www.sciencedirect.com/science/article/pii/S0099239916305258
http://www.iosrjournals.org/iosr-jdms/papers/Vol17-issue3/Version-18/A1703180106.pdf
https://pdfs.semanticscholar.org/36db/2dd0a7f3ed7b5edda016f702168fc66fd9c0.pdf
https://www.ncbi.nlm.nih.gov/pubmed/10530266

Instances where selected sources appear:

13

ANNEXURE-III

INFORMED CONSENT:

I have been informed regarding the procedure (Root Canal Treatment) done by the operating dentist in order to treat my dental pain, also I am being informed about the pulpal samples taken during the procedure which has been really safe. By signing below, I agree that my dentist has answered to all my questions and that I understand and accept the necessity as well the procedure and its benefits obtained through this.

Date: 15.5.2018 **Patient's name:** [illegible]

Place: Madurai **Signature:** [illegible]

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ANNEXURE

ஒப்புதல்அறிக்கை

நான்இந்தஆய்வுக்குறியகாரணங்களையும்,முறைகளையும்,இதிலுள்ளசவால்களும் எனக்குதெரிவிக்கப்பட்டுள்ளது, இந்தஆய்வில்எடுக்கும்அனைத்து வியரில் இருக்கும் இன்னைபுதிசுகல்உம்அறிந்தேன்.அதைநான்புரிந்துகொண்டேன்.இந்தஆய்வை பற்றியகேள்விகளைகேட்டுபதிலைஅறிந்துகொண்டேன்.நான்இந்த ஆய்வில்பங்குகொள்ளமுழுமனதுடன்சம்மதிக்கிறேன்.

தேதி: 15.5.2018 பங்குபெறுபவர்பெயர்: செல்வி

இடம்: மதுரை கையொப்பம்: செல்வி

ஆய்வாளரின்அறிக்கை

நான்இந்தஆய்வைபற்றிவாய்மொழியாகபங்குகொள்பவருக்கு, ஆய்வைபற்றியகாரணங்களையும், முறைகளையும், சவால்களையும்விளக்கியுள்ளேன். இதற்காக எடுக்கும் அனைத்து எச்சரிக்கையும் விளக்கியுள்ளேன். பங்குபெறுபவர்இதைபுரிந்துகொண்டிருப்பார்என்றுநான்நம்புகிறேன்.

தேதி: 15.5.2018 பங்குபெறுபவர்பெயர்: Dr. J. Evangeli

இடம்: Madurai. கையொப்பம்: Evangeli